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Monitoring of Viral-Induced Cell Death Using Real-Time Cell Analysis

David Thirkettle-Watts and Penny Gauci

Land Division
Defence Science and Technology Group

DST-Group-TR-3315

ABSTRACT

The TCID₅₀ is a measure of infectious viral titre in a sample. Traditionally this assay has been carried out using a microscopy based assay. This assay is highly subjective and relies on the correct identification of cytopathic effect (CPE) by eye. To ascertain whether the xCELLigence real-time cell analysis (RTCA) platform could be used to deliver a more objective and robust measure of TCID₅₀, a comparative study of the two methods was carried out. Two viruses were analysed, Gan Gan and Holmes Jungle, members of family *Bunyaviridae* and *Rhabdoviridae* respectively. The RTCA method gave results very close to the traditional method and was significantly quicker and easier to analyse. The RTCA method also has the potential to provide additional useful information about the viral infection of cells.

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Monitoring of Viral-Induced Cell Death Using Real-Time Cell Analysis

Executive Summary

To develop a *de novo* pathogen identification capability, the virology team in Land Division has been utilising next-generation DNA sequencing to sequence and characterise novel Australian viruses. Due to advances in the speed, throughput and cost of next-generation sequencing, this technology can be applied for the identification of viruses and other pathogens from complex samples, such as clinical and environmental samples. DST is currently developing methods to apply this technology to identify viruses directly from serum. A proof of concept experiment using serum samples spiked with known viruses will aid in determining appropriate procedures and detection limits. To proceed with this project, it is essential to determine the concentration of the viral stocks that will be used to spike the samples.

Two viruses previously sequenced and characterised by DST Group have been selected for this project. The first, Gan Gan virus (GGV; family *Bunyaviridae*) has been associated with an acute endemic polyarthritides-like illness in humans. The second, Holmes Jungle virus (HOJV; family *Rhabdoviridae*) has been detected in hospitalised patients. One measure of virus concentration is through the determination of the 50% tissue-culture infectious-dose (TCID₅₀). This measure describes the amount of virus that is required to produce an infection morphology, the cytopathic effect (CPE), in 50% of the population of cultured cells. Traditionally this work is carried out using an end point dilution assay; infecting cultured cells with serially diluted aliquots of virus which, after a fixed period of time, are scored for CPE by manual observation. This assay is highly subjective and relies on the correct identification of cytopathic effect (CPE) by eye.

In this study, the xCELLigence real-time cell analysis (RTCA) platform was evaluated as to whether it could be used to deliver a more objective and robust measure of TCID₅₀. The xCELLigence platform measures the electrical signal exerted by cultured cells grown on a microelectronic biosensor on the base of a 96-well cell culture plate. Using this instrument, it is possible to dynamically monitor attributes such as cell attachment, proliferation and morphology. By measuring the change in impedance produced by infected cells over time, a process such as the formation of CPE can be observed.

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A comparative study of the two methods was carried out. The RTCA method gave results very similar to the traditional method and was significantly quicker and easier to analyse. The RTCA method also has the potential to provide other information about the viral infection of cells, for example, the time-line of infection and viral specificity assays. It may also be highly useful for viruses which do not grow well in cell culture or do not produce easily discernible CPE.

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Authors

David Thirkettle-Watts

Land Division

*David Thirkettle-Watts completed a BSc (Hons) in molecular genetics and molecular biology at the University of Western Australia (UWA) and a PhD in 2004. His PhD research involved the characterisation of the promoter regions of a family of soybean genes using molecular and transgenic techniques. Following this, David joined a research lab at the department of Plant Biology, UWA where he led a research project describing transcription factors and other DNA binding proteins which interacted with the mitochondrial DNA of the plant *Arabidopsis thaliana*. After moving to Melbourne, David joined DST Group in 2008 where he has worked on a diverse range of projects including the assessment of bio-detection assays, genetic marker discovery in castor bean and organophosphate toxicity. His current work includes the characterisation of cellular responses to chemical agents and the establishment of an in vitro toxicology pipeline for hazard assessment and medical countermeasure development.*

Penny Gauci

Land Division

Penny Gauci graduated from the University of Melbourne with a B Sc in 1992. After working as a technical officer at RMIT University, she joined DST Group in 1998. She initially worked in the AMBRI ion channel switch (ICS) biosensor program before joining the DNA vaccine program in 2001, producing vaccine candidates for western equine encephalitis virus. She has worked on the Virology program since late 2009 and has been involved in the identification and characterization of unknown rhabdoviruses and bunyaviruses.

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Abbreviations

ADF	Australian Defence Force
CI	Cell Index
CPE	Cytopathic effect
DI	Defective interfering particle
DNA	Deoxyribonucleic Acid
DST	Defence Science and Technology
FCS	Foetal calf serum
GGV	Gan Gan virus
HOJV	Holmes Jungle virus
IPNV	Infectious pancreatic necrosis virus
NGS	Next generation sequencing
PCR	Polymerise Chain Reaction
RTCA	Real-time cell analysis
TCID ₅₀	50% tissue-culture infectious-dose

1. Introduction

The virology team in Land Division has sequenced and characterised the genomes of several novel Australian viruses from a historical collection shared by Berrimah Veterinary Laboratories, Darwin, NT (Gauci *et al.* 2016; Gauci *et al.* 2015a; Gauci *et al.* 2015b; McAllister *et al.* 2014; Gubala *et al.* 2016 (under review)). This has primarily been achieved using next-generation sequencing (NGS) techniques. NGS and other sequencing technologies in development could be useful tools for identifying viruses from in biological and environmental samples. A proof of concept experiment, where spiked samples are processed using in-house sequencing pipelines, will aid in determining appropriate procedures and detection limits. To proceed with this project, it is essential to determine the concentration of the viral stocks that will be used.

Two viruses previously sequenced and characterised by DST Group have been selected for this project. These represent two viral families (*Bunyaviridae* and *Rhabdoviridae*) important to human health and represent different genome configurations (segmented and non-segmented, respectively). The first, Gan Gan virus (GGV) of family *Bunyaviridae*, was first isolated from a pool of *Aedes vigilax* mosquitoes South West of Nelson Bay, NSW in 1970 (Gard *et al.* 1973). Complement fixation testing and genomic characterisation confirmed that it belongs to the Mapputta serogroup within the genus *Orthobunyavirus* (Gauci *et al.* 2016; Marshall *et al.* 1980). This virus has been associated with an acute endemic polyarthritis-like illness in humans (Boughton *et al.* 1990). The second, Holmes Jungle virus (HOJV) of family *Rhabdoviridae* was isolated from *Culex annulirostris* mosquitoes near Darwin, Northern Territory, in 1987 and was detected in hospitalised patients in the area (Weir 2002).

The concentration of virus is measured in terms of the infectious virus titre (50% Tissue Culture Infectious Dose; TCID₅₀) present in a sample. This measure describes the amount of virus that is required to produce a cytopathic effect (CPE) in 50% of the population of cultured cells. Traditionally this work is carried out using an end point dilution assay; infecting cultured cells with serially diluted aliquots of virus and, after a fixed period of time, scoring for CPE within a designated area by manual observation.

The manual scoring of CPE is highly subjective and time consuming. The ability to objectively monitor the viral infection of *in vitro* cells would allow for improvements in accuracy and throughput. This assay could have particular value for viruses or cell lines (e.g. insect lines) that produce poorly discernible CPE. Recent studies have shown that real-time cell analysis (RTCA) platforms such as the xCELLigence can be used to gather quantitative measurements of viral infectivity in real-time without the use of labels (Paprocka & Kęsy 2015; Teng *et al.* 2013; Tian *et al.* 2012).

The xCELLigence platform measures the electrical impedance exerted by cultured cells grown on a microelectronic biosensor on the base of a 96-well microplate. Using this instrument, it is possible to dynamically monitor attributes such as cell attachment, proliferation and morphology (Thirkettle-Watts 2016). By measuring the change in

impedance produced by infected cells over time, a process such as the formation of CPE is likely to be easily identified using this platform.

Two viruses were assayed using the RTCA platform to determine the suitability of this platform for the determination of TCID₅₀ values. The results from the RTCA platform were directly compared to the results from parallel infections for the manual determination of TCID₅₀ in a 96 well plate.

2. Methods

2.1 Cell Culture

BSR cells (a subclone of the baby hamster kidney BHK-21 cell line) were obtained from Berrimah Veterinary Laboratories, Darwin. Cell culture medium, foetal calf serum (FCS) and additives were purchased from Gibco (Thermo Fisher Scientific, Adelaide). Cells were maintained in Basal Medium Eagle medium supplemented with 10 mM HEPES, 80 U/ml Penicillin/Streptomycin, 2 mM Glutamine and 2.5% FCS and incubated in a humidified incubator at 37°C.

2.2 Virus Infection and TCID₅₀ Assay

Viruses were obtained from the Berrimah Veterinary Laboratories, Darwin, NT, Australia and had been previously prepared and stored at -80 C. Standard viral infections were carried out in 200 µl cultures. BSR cells were seeded at a density of 2×10^4 cells/well in 96 well plates and immediately infected with virus (this was designated "day 0"). Viral stocks were serially diluted and then 50 µl of the virus suspension applied to the cells. A 10 fold dilution series was used and the diluted titres designated 10^{-1} to 10^{-10} . There were eight replicate wells per dilution. Infected cells were compared to uninfected control cells. Visual scoring of CPE was carried out on day five post infection. TCID₅₀ values were determined by the method of Reed-Muench (Reed & Muench 1938).

2.3 Impedance Measurement

The xCELLigence system (ACEA Biosciences, San Diego) was used to measure changes in cellular impedance resulting from viral infection in 96-well E-Plates (Peters & Scott 2009). The E-Plate view was used to allow for the visual representation of the effect of the virus on the cells and the underlying cause of the change in impedance. The E-Plate has electrode arrays integrated into the bottom of the wells which allow for the measurement of the impedance conferred by cells growing on this surface. Changes in the measured impedance are defined by a unit-less cell index (CI). Viral infections were scaled down to 100 µl to take into account the reduced surface area of the 96 well E-Plate. Prior to the addition of cells to the E-Plate, a CI measurement was taken in the presence of 25 µl growth medium to determine the background CI value for each well. This was subtracted

from the subsequent CI values as they were collected following cell attachment. Cells were seeded at a density of 1×10^4 cells/well and infected with 25 μ l of viral suspension. The E-Plate was covered with an adhesive gas permeable membrane (Thermo Fisher Scientific, Adelaide) to ensure viral containment. Cultures were kept in the 37°C incubator for the duration of the experiments. The sampling frequency ranged between 15 min and four hours. For analysis, between six and eight replicates were used for each treatment per experiment.

3. Results

3.1 Optimisation of cell seeding density and growth conditions

In order to use the RTCA system to measure viral infectivity, there was a requirement to optimise the seeding density of the host cells and to ensure that the cells were able to grow efficiently when covered with the adhesive membrane. The membrane was a condition of use for the laboratory that the work was carried out in and not something absolutely required for this work.

Cell impedance was measured from each well of a 96-well e-plate seeded with varying densities of cells ranging from 1.25×10^3 to 6×10^4 cells/well. Two plates were run in parallel, one covered with the membrane and the other un-covered. Cell impedance measurements were taken over a period of 120 hours (Fig. 1). The cellular impedance increased over time as cells proliferated. The higher density of cells reached a plateau sooner, as would be expected. There was no difference between the growth curves between cells that were covered with the gas permeable membrane compared to those which were uncovered.

The manual observation of CPE is observed at 5 days post infection. At this time point, the impedance profile of cells seeded at a density of 1×10^4 cells/well reached its plateau. This was the density equivalent to that used for the traditional assay and this density was chosen to use for further experiments.

3.2 Viral infection

Based on the cell proliferation experiment, 1×10^4 cells were infected with serial ten-fold dilutions from neat to 10^{-10} of HOJV or GGV. Uninfected control wells were also included. The equivalent 96 well plates were also prepared in parallel to allow for the visual assessment of CPE. Infection with both viruses resulted in changes in the impedance profiles clearly indicative of viral infection.

3.2.1 Gan Gan Virus

The RTCA analysis of GGV infection was characterised by an increase in CI, peaking after approximately 10 hours, followed by a decrease in CI, plateauing at the baseline (Fig. 2A). The point at which the infection was evident was dependent on the viral dilution, indicating a correlation of the onset of CPE and the infectious viral load.

A determination of the number of wells showing CPE was made. At the 10^{-5} virus dilution, one well showed no sign of CPE at day 5, although CPE was observed shortly after this (Fig. 2B; light blue). It was also observed that at this level of infection, within a single titre of virus, the onset of CPE could occur at varying times (Fig. 2B). For cells infected with the 10^{-5} virus dilution, there were five distinct profiles showing CPE, CI peaking between 80 and 144 h. At 10^{-6} two wells showed signs of CPE at day 5 with a further well at 144 h. The CPE results from the impedance trace were confirmed microscopically (Fig. 2C).

The TCID₅₀ was determined from these results and compared to the values derived from the traditional microscopy method (Table 1). The results were comparable between the two methods with $1.12 \times 10^6/50\mu\text{l}$ from the RTCA method compared with $1.3 \times 10^6/50\mu\text{l}$.

3.2.2 Holmes Jungle Virus

The RTCA analysis of HOJV infection also showed changes in CI indicative of the viral infection of the cells. At low dilutions (below 10^{-3}), the impedance profile was similar to GGV, characterised by an increase in CI, peaking between six and twelve hours, followed by a decrease in CI, plateauing at the baseline (Fig. 3A). At dilutions of 10^{-3} and higher, the impedance profile did not indicate a return of CI to base-line levels implying that the morphology of these cells was different with the cells appearing to recover. This observation was confirmed microscopically where cells infected with neat virus showed a large number of cells with a live cell morphology (Fig. 3C). Similar to GGV, the point at which the infection was evident was dependent on the viral dilution, indicating a correlation of the onset of CPE and the infectious viral load.

A determination of the number of wells showing CPE was made. At the 10^{-5} virus dilution all wells showed signs of CPE at day 5. At 10^{-6} , three wells showed signs of CPE at day 5. Similarly to GGV, it was also observed that at this level of infection, within the same titre of virus, the onset of CPE could occur at varying times (Fig. 3B). For cells infected with a 10^{-6} virus dilution, there were three distinct profiles showing CPE, CI peaking between 70 and 110 h. The CPE results from the impedance trace were confirmed microscopically (Fig. 3C).

The TCID₅₀ was determined from these results and compared to the values derived from the traditional microscopy method (Table 1). The results were comparable between the two methods with $1.48 \times 10^6/50\mu\text{l}$ from the RTCA method compared with $1.3 \times 10^6/50\mu\text{l}$.

4. Discussion

The observation of CPE and its use in the quantification of infectious virus titres has long been used to determine lethal doses of viral samples. The major limitation of the traditional TCID₅₀ assay is its requirement for manual visual examination of each individual well to score infection (CPE). This can be time consuming and CPE can sometimes be difficult to determine.

The use of the xCELLigence RTCA platform to quantify the infectious virus dose was demonstrated through the use of BSR cells infected with either HOJV or GGV. The RTCA system makes use of three factors: cell number, morphology and adhesion to give rise to an impedance to the micro-electrical array on which the cells are grown. The viral infection of a cell results in morphological changes which are reflected in the change in CI. Any resulting cell death will affect both the adhesion and cell number, which is also reflected by a change in CI. The impedance profile of the infected cells in this experiment showed distinct phases which it is reasonable to assume will follow the course of the viral infection time-line; normal cell proliferation, viral infection resulting in a changed morphology and an increased CI lasting approximately 10 h and viral infection resulting in cell death likely through the activation of apoptotic pathways, reducing CI to base-line (Iranpour *et al.* 2016). This pathway could be confirmed by further study.

At very high viral titres, the impedance profiles and cell morphology of HOJV suggests that the infection of cells does not lead to cell death. This observation is most likely the result of defective interfering particles (DIs), which are incomplete non-infectious viruses. The formation of DIs at a high moiety of infection is a common characteristic of many viruses that contain negative sense single stranded genomes. The phenomenon is likely to be a mechanism used by the virus to slow down its replication, presumably to ensure the survival of the host and increase the spread of the virus to other hosts (Pathak & Nagy 2009; Thompson & Yin 2010).

The time at which cells exhibit CPE is proportional to the virus dilution (Fig. 4). For both viruses, the time to peak CI showed a linear relationship with the log of virus at dilutions below 10⁻⁴. At dilutions above this the number of virus particles present is presumably so low that the rate of cell infection becomes extremely variable as shown by the increased variability of the time to peak CI. As the viral load per well approaches the TCID₅₀ level, the infection timing becomes more variable as indicated by the increased range over which CPE is observed. For example, GGV at the 10⁻² dilution, all wells showed a peak CI at 44.25 h whereas at 10⁻⁵, the time to peak CI ranged from 74.25 h to 141 h with a mean of 93.8 h ± 22 h.

The results clearly show that the RTCA platform is an effective tool for the measurement and quantification of viral infection. The TCID₅₀ values determined by RTCA were in very close agreement with those determined using the visual observation of CPE via a microscope. The RTCA gives further advantage due to its ability to dynamically monitor viral infection during the whole growth period. It can provide information about the timing of infection and potentially other effects of viral infection on the cell not easily

identified by other methods. It provides a platform from which other virology research can be carried out, including viral neutralisation, infection kinetics and medical countermeasure development.

The developed method will be used in parallel with our in-house real-time PCR assays to assess the amount of virus that will be required to spike serum samples. These samples will be used for the development of next-generation sequencing methods for *a priori* identification of pathogens from clinical samples obtained from ill ADF personnel under DST's ADF endemic disease study. The developed method will enhance DST's detection and diagnostic capability.

5. References

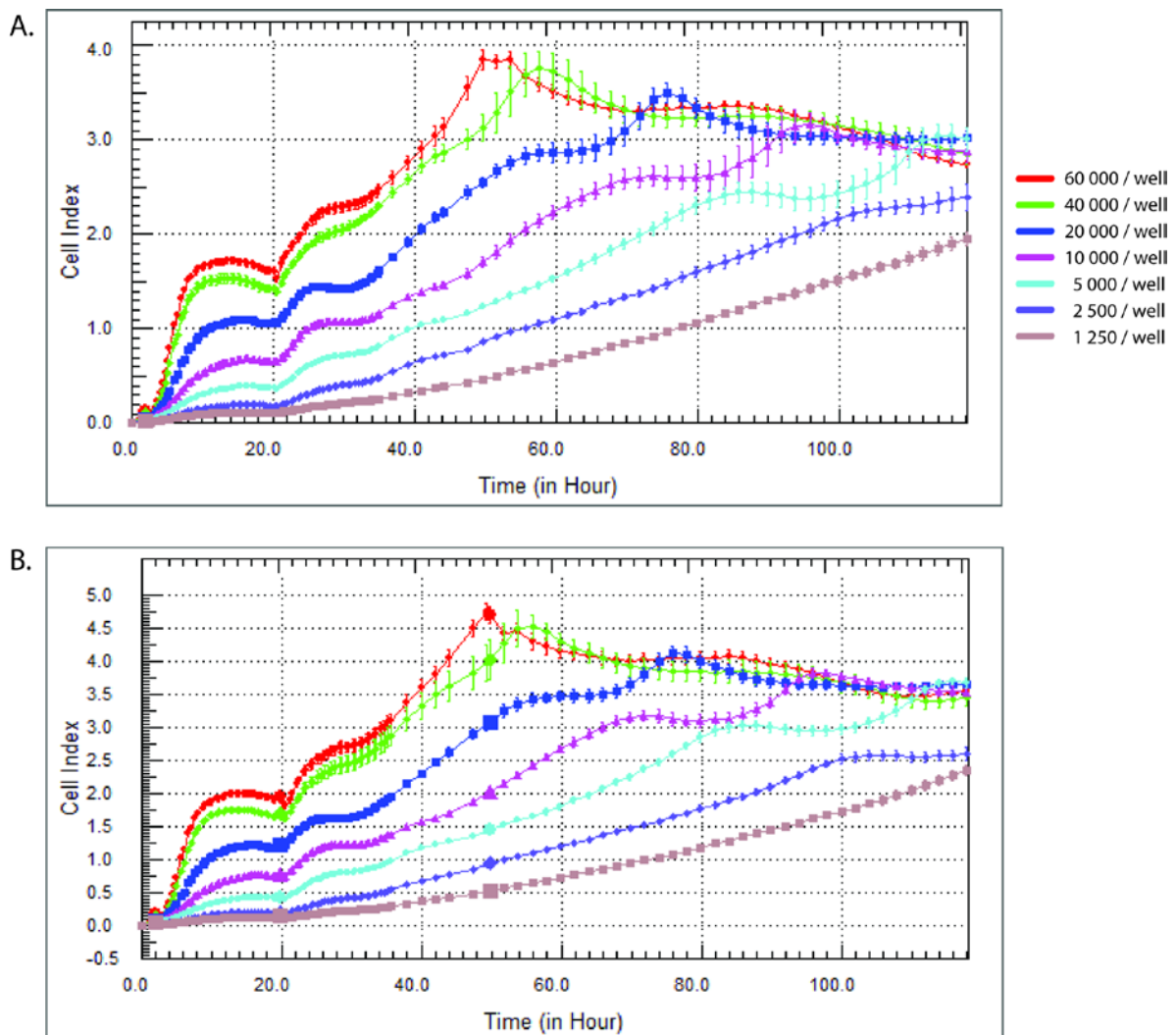
- Boughton, C. R., Hawkes, R. A., Naim, H. M. Arbovirus infection in humans in NSW: Seroprevalence and pathogenicity of certain Australian bunyaviruses. *Australian and New Zealand Journal of Medicine* 20 (1) 1990 51-55.
- Gard, G., Marshall, I. D., Woodroffe, G. M. Annually recurrent epidemic polyarthritides and Ross River virus activity in a coastal area of New South Wales. II. Mosquitoes, viruses, and wildlife. *American Journal of Tropical Medicine and Hygiene* 22 (4) 1973 551-560.
- Gauci, P. J., McAllister, J., Mitchell, I. R., Weir, R. P., Melville, L. F., Gubala, A. J. Genomic characterisation of Trubanaman and Gan Gan viruses, two bunyaviruses with potential significance to public health in Australia. *Virology Reports* 6 2016 1-10.
- Gauci, P. J., McAllister, J., Mitchell, I. R., Boyle, D. B., Bulach, D. M., Weir, R. P., Melville, L. F., Gubala, A. J. Genomic characterisation of three mapputta group viruses, a serogroup of Australian and papua new guinean bunyaviruses associated with human disease. *PloS one* 10 (1) 2015a e0116561.
- Gauci, P. J., McAllister, J., Mitchell, I. R., George, T. D. S., Cybinski, D. H., Davis, S. S., Gubala, A. J., St George, T. D. Hunter Island Group Phlebovirus in Ticks, Australia. *Emerging Infectious Diseases* 21 (12) 2015b 2246-2248.
- Iranpour, M., Moghadam, A. R., Yazdi, M., Ande, S. R., Alizadeh, J., Wiechec, E., Lindsay, R., Drebot, M., Coombs, K. M., Ghavami, S. Apoptosis, autophagy and unfolded protein response pathways in Arbovirus replication and pathogenesis. *Expert Reviews in Molecular Medicine* 18 2016 e1 (21 pages).
- Marshall, I. D., Woodroffe, G. M., Gard, G. P. Arboviruses of coastal South-Eastern Australia. *Australian Journal of Experimental Biology and Medical Science* 58 (1) 1980 91-102.
- McAllister, J., Gauci, P. J., Mitchell, I. R., Gubala, A. J., Boyle, D. B., Bulach, D. M., Weir, R. P., Melville, L. F., Davis, S. S. Genomic characterisation of Almpiwar virus, Harrison Dam virus and Walkabout Creek virus; three novel rhabdoviruses from northern Australia. *Virology Reports* 3 2014 1-17.
- Paprocka, G., Kęsy, A. Real-time replication of swine vesicular disease virus (SVDV) in cell culture systems in vitro. *Bulletin of the Veterinary Institute in Pulawy* 59 (4) 2015 457-462.

- Pathak, K. B., Nagy, P. D. Defective Interfering RNAs: Foes of Viruses and Friends of Virologists. *Viruses* (1999-4915) 1 (3) 2009 895-919.
- Peters, M. F., Scott, C. W. Evaluating cellular impedance assays for detection of GPCR pleiotropic signaling and functional selectivity. *J Biomol Screen* 14 (3) 2009 246-255.
- Reed, L. J., Muench, H. A simple method of estimating fifty per cent endpoints. *American Journal of Epidemiology* 27 (3) 1938 493-497.
- Teng, Z., Kuang, X., Wang, J., Zhang, X. Real-time cell analysis – A new method for dynamic, quantitative measurement of infectious viruses and antiserum neutralizing activity. *Journal of Virological Methods* 193 2013 364-370.
- Thirkettle-Watts, D. Impedance-based analysis of mu opioid receptor signaling and underlying mechanisms. *Biochemistry and Biophysics Reports* 6 2016 32-38.
- Thompson, K. A. S., Yin, J. Population dynamics of an RNA virus and its defective interfering particles in passage cultures. *Virology Journal* 7 2010 257-266.
- Tian, D., Zhang, W., He, J., Liu, Y., Song, Z., Zhou, Z., Zheng, M., Hu, Y. Novel, Real-Time Cell Analysis for Measuring Viral Cytopathogenesis and the Efficacy of Neutralizing Antibodies to the 2009 Influenza A (H1N1) Virus. *PloS one* 7 (2) 2012 e31965.
- Weir, R. (2002). *Classification and identification of viruses isolated from mosquitoes in the Northern Territory, 1982 - 1992, using a range of techniques. PhD thesis.* PhD thesis, Faculty of Medicine, University of Sydney, Australia.

6. Figures and Tables

Table 1 Summary of TCID₅₀ values.

Virus	TCID ₅₀ RTCA	TCID ₅₀ Microscopy
GGV	1.12 X 10 ⁶ / 50μl	1.3 X 10 ⁶ / 50μl
HOJV	1.48 X 10 ⁶ / 50μl	1.3 X 10 ⁶ / 50μl



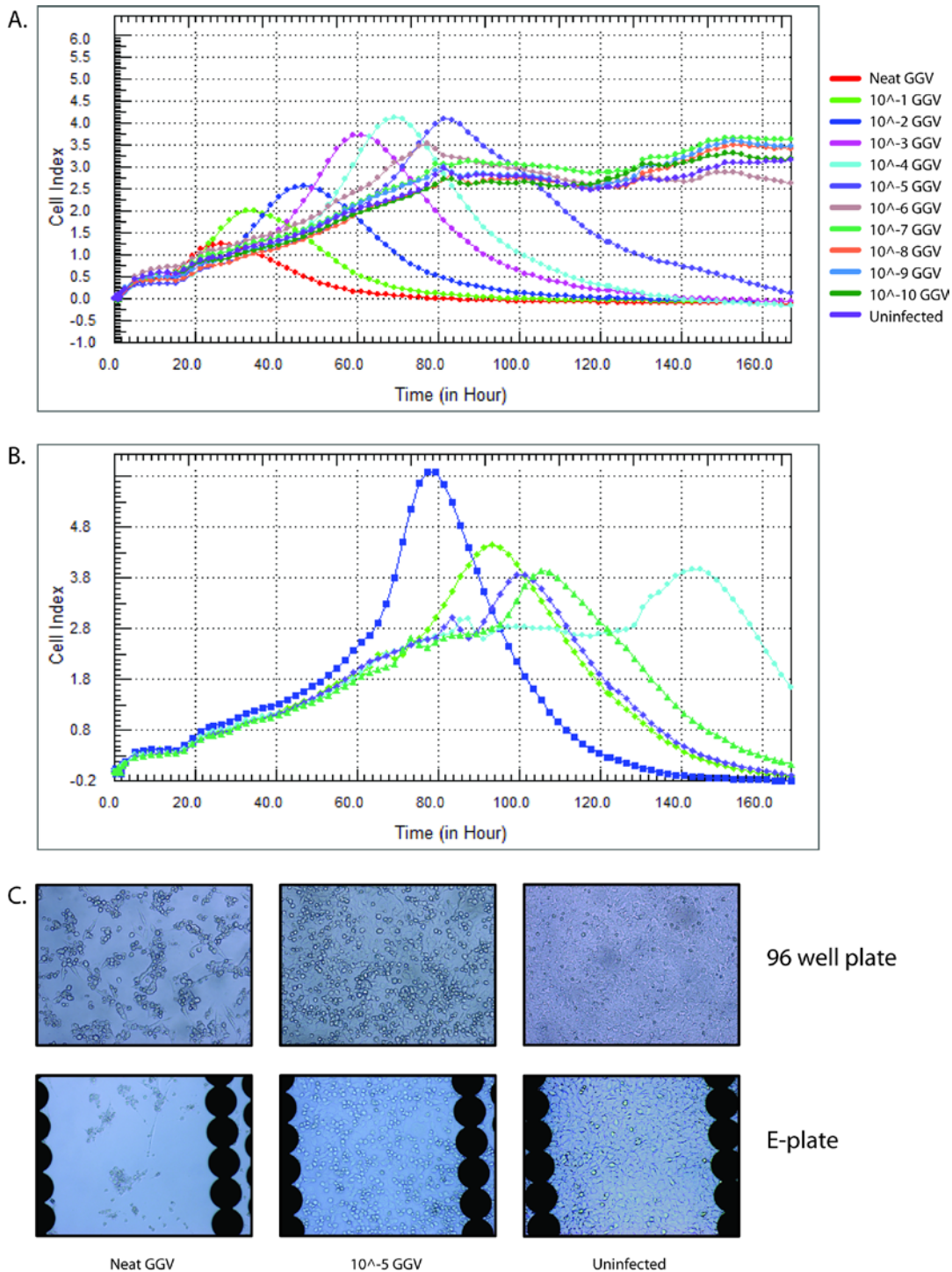


Figure 2. *Gan Gan* virus mediated cytopathogenicity. A) Real-time monitoring of BSR cells infected with a 1:10 dilution series of *Gan Gan* virus. The curve is an average of eight independent replicate wells. B) Difference in the timing of CPE development between wells with the same infectious load (10^{-5} dilution) C) The CPE of cells growing on either a standard 96 well plate or the E-plate infected with a high viral load (Neat), viral load close to the $TCID_{50}$ (10^{-5} dilution) or no virus (Uninfected).

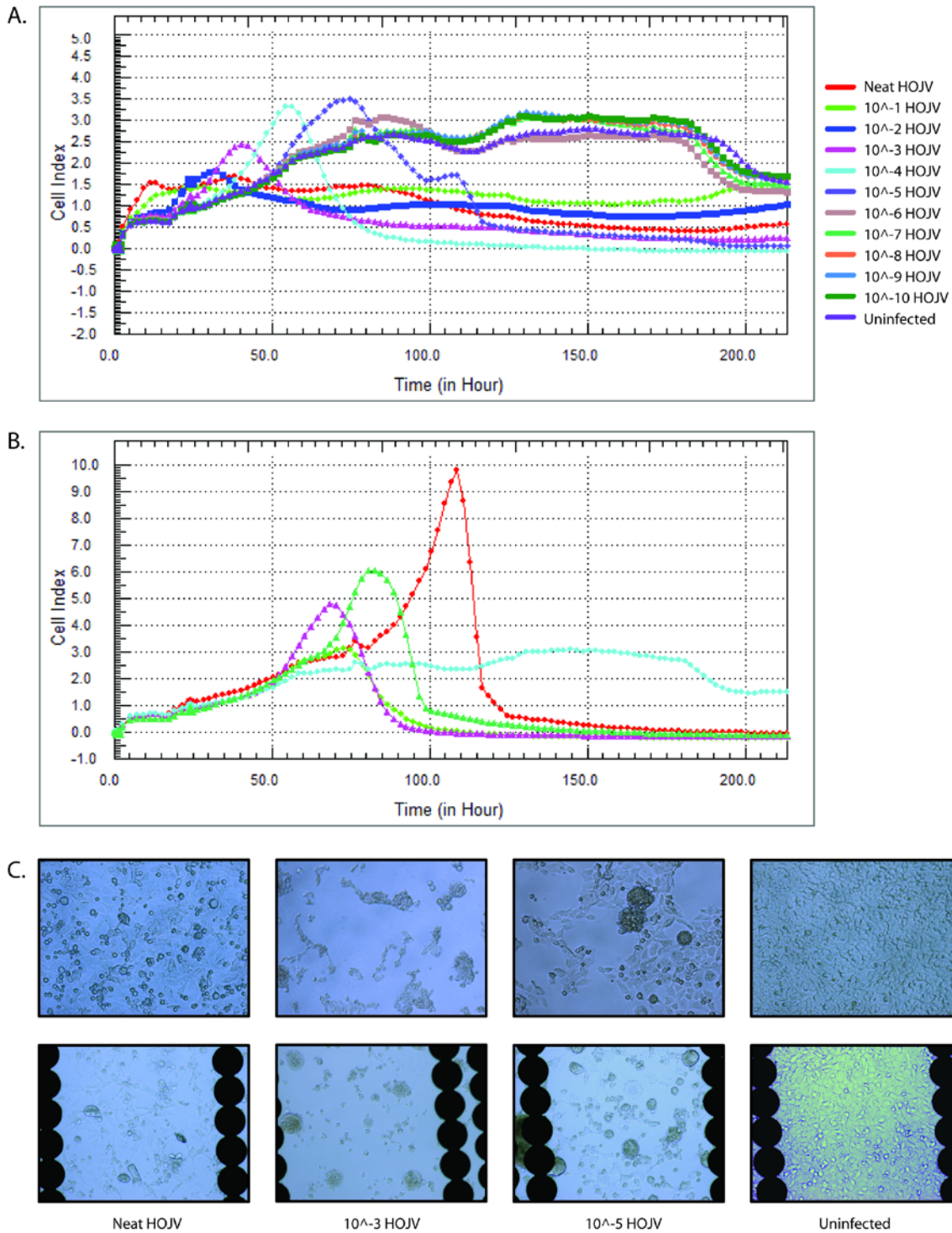


Figure 3. Holmes Jungle virus mediated cytopathogenicity. A) Real-time monitoring of BSR cells infected with a 1:10 dilution series of Holmes Jungle virus. The curve is an average of eight independent replicate wells. B) Difference in the timing of CPE development between wells infected with the same viral load (10^{-5} dilution) C) The CPE of cells growing on either a standard 96 well plate or the E-plate infected with a high viral load showing live cells (Neat), a high viral load with no live cells (10^{-3}), a viral load close to the $TCID_{50}$ (10^{-5} dilution) or no virus (Uninfected).

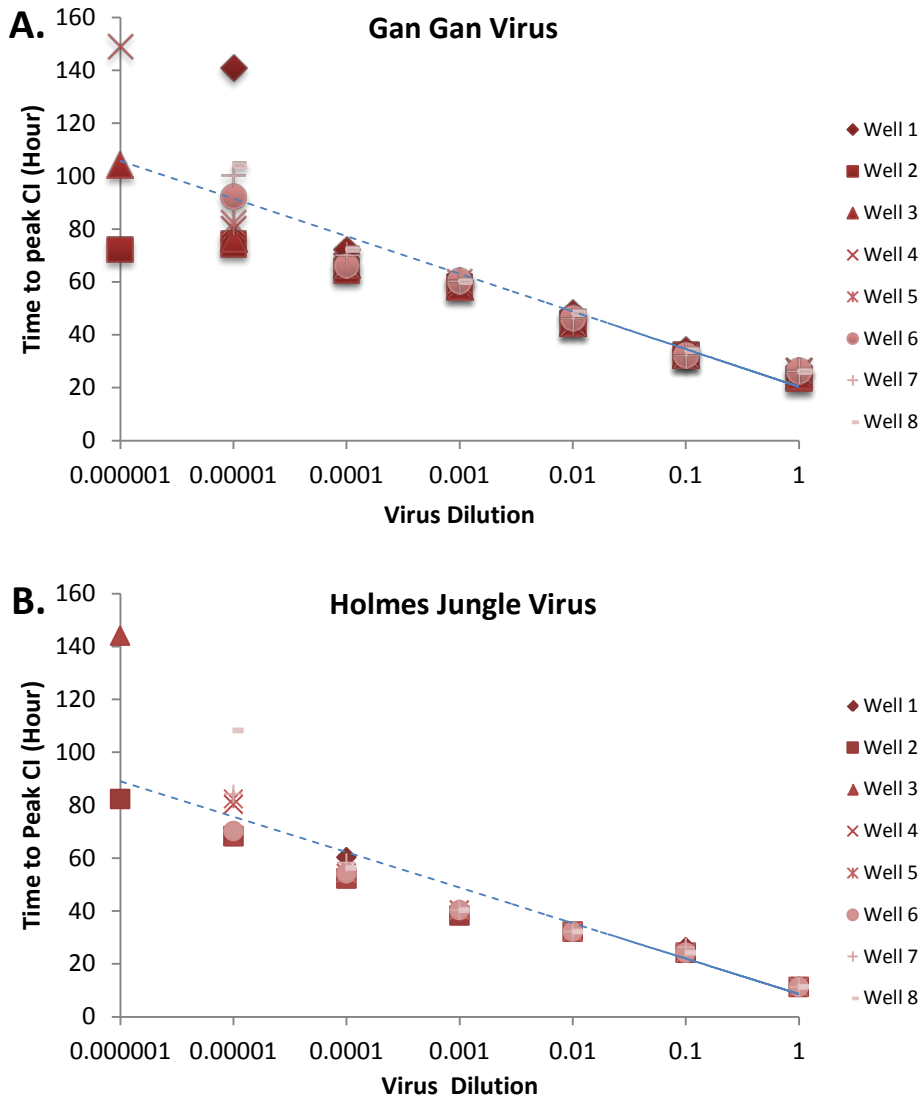


Figure 4. Relationship between viral titre and changes in impedance. The log of virus dilution was plotted against time to peak CI for each well of the E-plate. A) Gan Gan Virus. B) Holmes Jungle Virus. Dotted line represents line of best fit.

Appendix A: Reed-Muench TCID₅₀ table

Table A1 Calculation table used for determination of the TCID₅₀ using the Reed-Muench method.

	Number of wells infected with CPE (For an 8 well titration)							
Dilution	1+	2+	3+	4+	5+	6+	7+	8+
10 ⁻¹	0.62 ⁽⁴⁾	0.75 ⁽⁶⁾	0.87 ⁽⁷⁾	1 ⁽¹⁰⁾	1.12 ⁽¹³⁾	1.25 ⁽¹⁸⁾	1.37 ⁽²³⁾	1.5 ⁽³²⁾
10 ⁻²	1.62 ⁽⁴²⁾	1.75 ⁽⁵⁶⁾	1.87 ⁽⁷⁴⁾	2 ⁽¹⁰⁰⁾	2.12 ⁽¹³²⁾	2.25 ⁽¹⁷⁸⁾	2.37 ⁽²³⁴⁾	2.5 ⁽³¹⁶⁾
10 ⁻³	2.62 ⁽⁴¹⁷⁾	2.75 ⁽⁵⁶²⁾	2.87 ⁽⁷⁴¹⁾	3 ⁽¹⁰⁰⁰⁾	3.12 ⁽¹³¹⁸⁾	3.25 ⁽¹⁷⁷⁸⁾	3.37 ⁽²³⁴⁴⁾	3.5 ⁽³¹⁶²⁾
10 ⁻⁴	3.62	3.75	3.87	4	4.12	4.25	4.37	4.5
10 ⁻⁵	4.62	4.75	4.87	5	5.12	5.25	5.37	5.5
10 ⁻⁶	5.62	5.75	5.87	6	6.12	6.25	6.37	6.5
10 ⁻⁷	6.62	6.75	6.87	7	7.12	7.25	7.37	7.5
10 ⁻⁸	7.62	7.75	7.87	8	8.12	8.25	8.37	8.5
10 ⁻⁹	8.62	8.75	8.87	9	9.12	9.25	9.37	9.5
10 ⁻¹⁰	9.62	9.75	9.87	10	10.12	10.25	10.37	10.5

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17. CITATION IN OTHER DOCUMENTS Yes				
18. RESEARCH LIBRARY THESAURUS RTCA Virology TCID50 Real-Time Cell Analysis Virus				
19. ABSTRACT The TCID50 is a measure of infectious viral titre in a sample. Traditionally this assay has been carried out using a microscopy based assay. This assay is highly subjective and relies on the correct identification of cytopathic effect (CPE) by eye. In order to ascertain whether the xCELLigence real-time cell analysis (RTCA) platform could be used to deliver a more objective and robust measure of TCID50, a comparative study of the two methods was carried out. Two viruses were analysed, Gan Gan and Holmes Jungle viruses, members of family Bunyaviridae and Rhabdoviridae respectively. The RTCA method gave results very close to the traditional method and was significantly quicker and easier to analyse. The RTCA method also has the potential to provide other information about the viral infection of cells which if explored further could be useful.				

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